Journal of Experimental Agriculture International

43(5): 7-16, 2021; Article no.JEAI.69727
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Rooting of *Schinus terebinthifolia* Raddi Cuttings as a Function of the Preparation Method and Indolebutyric Acid Concentrations

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DMS, NPF, SSB Conceptualized the article. Data acquisition by DMS, NPF, JJOG. Data analysis and interpretation by authors DMS, SSB, NPF, JJOG, LM. Writing of the manuscript by authors DMS, SSB, NPF, JJOG, RFA, APCGB, LM. Supervision by author SSB. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2021/v43i530685
Editor(s):
(1) Prof. Rusu Teodor, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
Reviewers:
(1) Arpita Roy, Sharda University, India.
(2) Phillip Minnaar, Agricultural Research Council (ARC), South Africa.
Complete Peer review History: http://www.sdiarticle4.com/review-history/69727

Short Research Article

Received 19 April 2021
Accepted 29 June 2021
Published 07 July 2021

ABSTRACT

Schinus terebinthifolia Raddi, popularly known in Brazil as pink pepper or aroeira, has become a new source of agricultural exploitation for some farmers, notably located in the north of the state of Espírito Santo, the largest producing region in the world. Once it was a species that has been historically exploited in an extractive manner, where only what nature offers is explored, the need for the generation of scientific knowledge to understand its genetic potential in all agronomic areas is evident, starting with the large spread in scale of this species. Therefore, the objective of the work was to study the effects of indolebutyric acid (IBA) on the physiology and quality of seedlings produced by cuttings of the species *Schinus terebentifolia* Raddi under different concentrations of this hormone and different ways of application. The experiment design was a factorial in randomized blocks, in the 2x7 arrangement, the first being composed of two forms of preparation of the hormone

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(dilution in acetone or in water) and the second composed by 7 concentrations of IBA (0 mg/L; 625 mg/L; 1250 mg/L; 2500 mg/L; 3000 mg/L; 3750 mg/L and 5000 mg/L) with 5 blocks and 5 plants in each treatment. The addition of IBA favored the development and physiological aspects of the seedlings, produced from the hormone prepared with both, water and acetone. The quality of seedlings induced with IBA diluted in water was compromised with the addition of the hormone, whereas in seedlings treated with IBA diluted in acetone, the estimated dose of 1750 mg/L of IBA, promoted the better quality index (IQD) of the seedlings.

Keywords: Aroeira; indolebutyric acid; vegetative propagation; forest.

1. INTRODUCTION

A native plant from Brazil, *Schinus terebentifolia* Raddi, popularly known as aroeira, is a species belonging to the Anacardiaceae botanical family and has high ecological distribution, and is found in the entire Brazilian coast from Restinga to the tropical forests [1,2].

Brazil holds an important position in the production of aroeira, with the emphasis on the northern region of Espírito Santo and, in particular, in the county of São Mateus, which is the largest producer and exporter of fruits of this species to international markets [3]. Despite its importance, in general, its exploitation is extractive and predatory, which is contributing to the natural depletion of this species [4]. In this scenario, the development and improvement of the systematic cultivation of aroeira is important for the success of the production chain as well as for the conservation of this species.

The ways in which this species is propagated is reported in the literature, both in vegetative form and in the seminal way with a high degree of germination, which allows for the rapid obtaining of suitable seedlings for planting [4]. Due to the problems of seed propagation, among them, the unevenness of seed germination and seedling development and the high genetic variability, which can be detrimental to the productivity of plantations, therefore vegetative propagation techniques have become an alternative to overcome these difficulties [5,6,7].

Among the most used vegetative propagation methods in the production of seedlings of plants, including aroeira, cuttings are mostly used [6]. The success of this technique depends on the roots of the cuttings, which can be affected by endogenous (hormonal and nutritional balance of the mother plant, and endogenous auxin concentration) and exogenous substrate used, (environmental conditions) [8,9,10].

Slow growth is one of the difficulties to overcome in the production of forest seedlings, especially of species classified as late or climax, as is the case of aroeira [11]. In this sense, the treatment of cuttings with plant regulators, mainly auxins, has often been used to assist in the production of quality seedlings. Among the auxins, indolebutyric acid (IBA) rooting hormone is the most used for rooting cuttings [12].

Indolebutyric acid can be used both in powder or in liquid form, however the solid form promotes a longer exposure of the hormone in the target tissues and can enable more favorable responses [13]. For the preparation of different concentrations of IBA, regardless of the form of application, an organic solvent is usually used to improve its dilution, since auxins have low water solubility making it difficult to obtain a homogeneous mixture [14,15].

Once aroeira standing out on the international stage for the trade of its fruits used as condiments, applied research becomes necessary to solve problematic issues of this species, favoring migration from predatory extractivism to sustainable cultivation techniques, and seeking to improve the cultivation of aroeira to extract its fruits. Thus, the objective of this work was to study the effects of indolebutyric acid (IBA) on the physiology, development and quality of plants produced by cuttings of the species *Schinus terebentifolia* Raddi under different concentrations of IBA hormone and different applications.

2. MATERIAL AND METHODS

The experiment was carried out in a commercial nursery located in the county of Jaguaré - ES, with geographic coordinates 18 ° 54 ’23” south latitude and 40º 04 ’31” west longitude, from August, 2017 to August, 2018. The region's climate is Tropical Aw, according to the Köppen climate classification, the average annual temperature is 23.5°C and the average annual precipitation is 1291 mm [16].

The experimental model was adopted in the form of a factorial in randomized blocks in a 2 x 7 arrangement. The first factor being two forms of preparation of the indolebutyric acid hormone (IBA) for application via powder (preparation with dilution of IBA in pure acetone and preparation with dilution IBA in water) and the second factor composed of 7 concentrations of IBA. The experiment had 5 blocks and 5 plants in each treatment. The adopted treatments are explained in Table 1:

Table 1. Details of Factor 2 levels with the doses used of the IBA hormone for each form of preparation (Factor 1)

Treatments	Doses
T1	pure powder (undiluted IBA)
T2	625 mg/L IBA
T3	1250 mg/L IBA
T4	2500 mg/L IBA
T5	3000 mg/L IBA
T6	3750 mg/L IBA
T7	5000 mg/L IBA

* It is used arbitrarily by producers in the northern region of the Brazilian state, Espírito Santo, at the dosage of 2500 mg/L of IBA.

The IBA hormone was diluted in water or acetone in the dosages established in Table 1 and then mixed with industrial talc powder for application of the treatments. Plant cuttings were planted in trays in tube of 100 cm^3, filled with commercial substrate Carolina®, composed of Sphagnum Peat (peat moss), expanded Perlite, expanded Vermiculite, and carbonized rice husks.

The treatments were carried out in a commercial nursery using aroeira cuttings for 150 days, with relative air humidity of between 70 to 80% and automatic irrigation by nebulizer carried out every 5 minutes for a period of 10 seconds, keeping the substrate always close to field capacity, that is, close to the maximum amount of water it can store. The cutting cultivation environment of this experiment followed the same conditions as what the commercial seedling nurseries produce.

At 150 days after cutting, physiological and morphological evaluations were performed on the plants. Using a Multiplex fluorometer (Force-A, França) with multiple sources of light excitation (ultraviolet, blue, green and red) in order to estimate nitrogen balance indices (NBI_G + NBI_R), flavonoids and chlorophyll total (SFR_G + SFR_R). Other parameters evaluated were the green color index, using a Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502 Plus, Konica Minolta, Japão) and leaf area, determined by a Leaf Area Meter Model LI-3100C (Li-Cor, United States).

After the physiological analyzes, evaluations of height measurement (standardized from the neck to the apical meristem) and measurement of the stem diameter (measured with a caliper on the plant's neck) were made, gravimetric assessments of shoot dry matter (SDM), root dry matter (RDM) and total dry matter mass (TDM) were also performed. To obtain the dry matter, a forced circulation drying oven (Model MA 035/5, Marconi, Brazil) at 72 ° C for 72 hours was used where after the dried sample was weighed on a precision analytical balance (Model AUY 220, Shimadzu, Japão). From the seedling development data, Dickson's quality index was calculated using equation 1, proposed by Dickson *et al*. [17]:

$$IQD = \frac{TDM}{\frac{H}{D} + \frac{SDM}{RDM}} \qquad (1)$$

Where:

IQD = Dickson´s Qualidade Index.
TDM = Total Dry Matter Mass.
H = Height.
D = Diameter.
SDM = Shoot Dry Matter.
RDM = Root Dry Matter.

The data obtained were submitted to analysis of variance by the F-test ($p < 0.05$) with the aid of the R core team software [18] and the means compared by the Tukey test at 5% probability. When a significant effect was observed, polynomial regression models were adjusted that best explained the variables.

3. RESULTS AND DISCUSSION

With the exception of the stem diameter variable, significant interactions were found between the factors studied for all assessed variables, that is, the way in which IBA (water or acetone) is prepared interferes with most of the evaluated characteristics (Tables 2 and 3).

Cuttings subjected to the preparation of the IBA with dilution in acetone were superior to those made with IBA diluted in water in most of the characteristics evaluated in this study (Tables 2 and 3), notably in relation to the quality index (IQD), because takes into account important variables to plant development (TDM, H, D, SDM and RDM). One hypothesis is IBA low water solubility [14] resulting in less homogeneous mixtures. That is why , an organic solvent (acetone) was used in this study to increase the solubility, therefore, the dilution of the hormone in acetone for later addition of talc, may have facilitated the hormonal action in the aroeira cuttings. It made the IBA mixture more homogeneous and the cuttings were subjected to the correct concentration of each treatment studied.

Table 2 . Characteristics of development and quality of aroeira plants propagated by cuttings according to the form of preparation of IBA (water or acetone)

Treatment	Height (cm)	Leaf area (cm^2)	SDM (g)	RDM (g)	TDM (g)	IQD
Water	19.15 a	8.63 b	1.65 a	0.31 b	1.96 a	0.22 b
Acetone	13.20 b	12.69 a	1.38 b	0.36 a	1.74 b	0.28 a
Average	16.18	10.66	1.52	0.34	1.85	0.25
CV (%)	**24.06**	**19.15**	**19.66**	**13.58**	**15.7**	**17.46**

Means followed by different letters in a column, differ statistically from each other by the Tukey test at the level of 5% (p <0.05)

Table 3. Physiological characteristics of aroeira plants propagated by cuttings according to the method of preparation of IBA (water or acetone)

Treatment	Nitrogen Balance (NBI_G+NBI_R)	Flavonoids	Total chlorophyll (SFR_G+SFR_R)	SPAD
Water	0.75 b	0.55 a	2.14 b	37.47 b
Acetone	0.83 a	0.53 a	2.44 a	46.75 a
Average	0.79	0.54	2.29	42.11
CV (%)	12.4	11.92	13.82	8.39

Means followed by different letters in a column, differ statistically from each other by the Tukey test at the level of 5% (p <0.05)

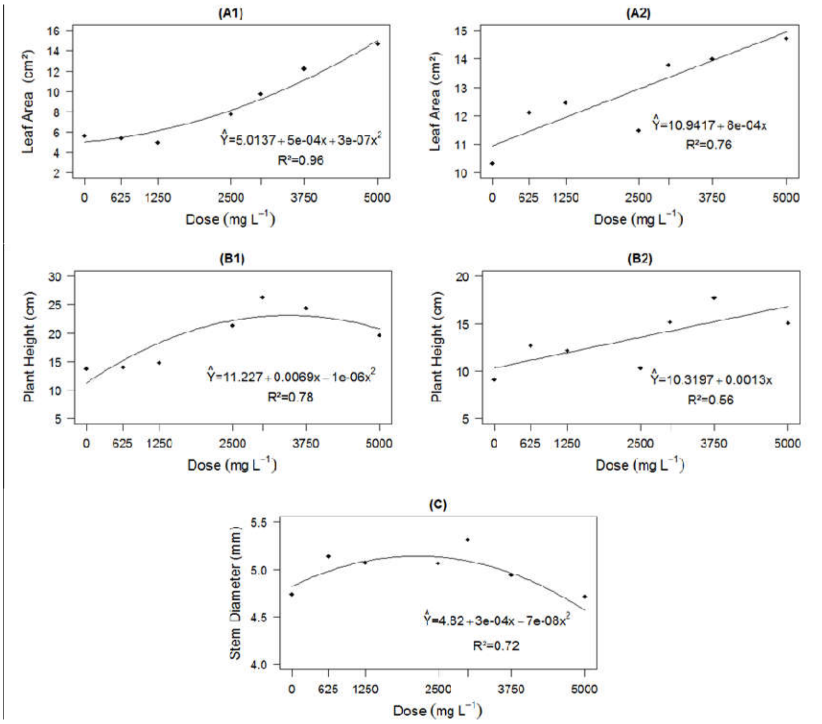

Fig. 1. Leaf area (A), plant height (B) and stem diameter (C) of cuttings produced with IBA diluted in water (1) or acetone (2) from cuttings at 150 days of age depending on the hormone doses applied

For the effects of doses within the factors, after unfolding in quadratic regression, the effects found in the stem diameter variable indicated that the highest estimated value was 5.14 cm with 2142.86 mg / L of IBA (Fig. 1-C). For this characteristic, as there was no interaction between the factors, to reach this maximum value, it did not matter whether the solvent used to dilute the IBA was water or acetone.

This reduction in stem diameter after the estimated dosage of 2142.86 mg / L of IBA, can be explained by the toxic effect caused by IBA when used in high concentrations. According to Lone [19], the use of exogenous auxin, applied in the cuttings, the rooting increases to a maximum value where, after which, any increase in auxin has an inhibitory effect, therefore, possibly the higher IBA doses caused damage to the stem of the plants, as well as in others evaluated parameter in this experiment. High concentrations of IBA can cause changes in enzyme activity and cofactor content and lead to the establishment of an endogenous hormonal imbalance affecting rhizogenesis and, consequently, the growth of the aerial part of the plants [20,21].

The leaf area of cuttings obtained with IBA diluted in water showed a quadratic behavior, but with a tendency similar to that of the leaf area found in cuttings prepared with IBA diluted in acetone, which had a linear and increasing behavior with the increase of IBA doses. For both plants, obtained with IBA diluted in water and in acetone, the increase in IBA concentration favored an increase in the leaf area of the plants (Fig. 1-A1 and 1-A2). This increase may have been favored by the activity of the root system of plants with the increase of IBA concentration, since the use of IBA in the production of plants, increases rooting and favors the increase in the volume and length of the roots producing a system with a vigorous root, which allows an increase in the absorption of water and nutrients and, consequently, in the gain of leaf material and aerial part [22,23].

When analyzing the plant height variable, it was observe that the cuttings submitted to IBA diluted in water presented a quadratic projection in which the highest estimated value was 23.13 cm at the dose of 3450 mg/L of IBA. From that value, the plant height decreased with the increase in IBA doses (Fig. 1-B1). As already described, IBA, in high concentrations, caused damage to the plant, which, this is possibly the cause of the reduction in plant height when using doses above the optimum point found. In the cuttings in which the IBA diluted in acetone was used, a linear and increasing response was observed with the increase in IBA doses. Using the adjusted linear regression model, it was estimated that the dose of 5000 mg/L reached approximately 16.57 cm per cutting, representing an increase of 60.56% in relation to the treatment without the application of IBA (Fig. 1-B2).

According to Ferreira et al. [13] auxin crystals are insoluble in water. Therefore, dilution of IBA in water may have made it difficult to obtain a homogeneous mixture. Therefore, the heterogeneity of the mixture may have caused the IBA to act more concentrated in the cuttings in the characteristic plant height, causing more noticeable damage in the higher concentrations of the hormone, which favored the phytotoxic effect for this high dilution vehicle concentrations. The increase in the solubility of IBA when diluted with acetone, possibly favored the obtaining of mixtures with homogeneous concentrations and, therefore, the hormone acted in a more balanced way in plants prepared in IBA diluted in acetone at the parameter plant height.

This same explanation can be given for the variables shoot dry matter (SDM), flavonoid index and total chlorophyll index (SFR_G + SFR_R), since the cuttings showed response patterns similar to the plant height variable. The shoot dry matter (SDM), the flavonoid index and the total chlorophyll index (SFR_G + SFR_R) of the cuttings submitted to treatment with IBA diluted in water, obtained increasing values up to the maximum point estimated in the doses of 3333,33 mg/L, 2500 mg/L and 2857.14 mg/L of IBA respectively. It was possible to visualize a reduction from these points (Fig. 2-B1, 3-B1 and 3-C1). In the cuttings prepared with IBA prepared with acetone, all of the above variables tended to be higher with the increase in IBA doses (Fig. 2-B2, 3-B2 and 3-C2).

The root dry matter (RDM) and total dry matter mass (TDM), when using the IBA diluted with water, obtained values up to 0.37 g and 2.21 g in the doses of 1500 mg/L and 2857.14 mg/L of IBA, respectively (Fig. 2-A1 and 2-C1). When the IBA diluted with acetone was used, the root dry matter mass (RDM) had the highest estimated value at the dose of 1666.67 mg / L with 0.42 g. The total dry matter mass (TDM) at the dose 4000 mg/L was 2.07 g (Fig. 2-A2 and 2-C2). The decrease in these parameters, after reaching the maximum value described, may also have been favored by the toxic effect of IBA.

It was noted that the Dickson Quality Index (IQD) had a linear and decreasing response pattern with increasing doses of the hormone when the aroeira cuttings were treated with IBA diluted with water. Using the adjusted linear regression model, it was estimated that the highest IQD was 0.27 in the treatment without IBA (Fig. 2-D1). This shows that even with the significant increase of some characteristics, such as the height of the plants, SDM and TDM, it should be noted that the advent of increased concentrations of IBA diluted in water induced a reduction in the quality of cuttings. These effects could also be observed in the physiological characteristics, in which the chlorophyll levels were below those observed in the leaves of cuttings induced by IBA diluted in acetone.

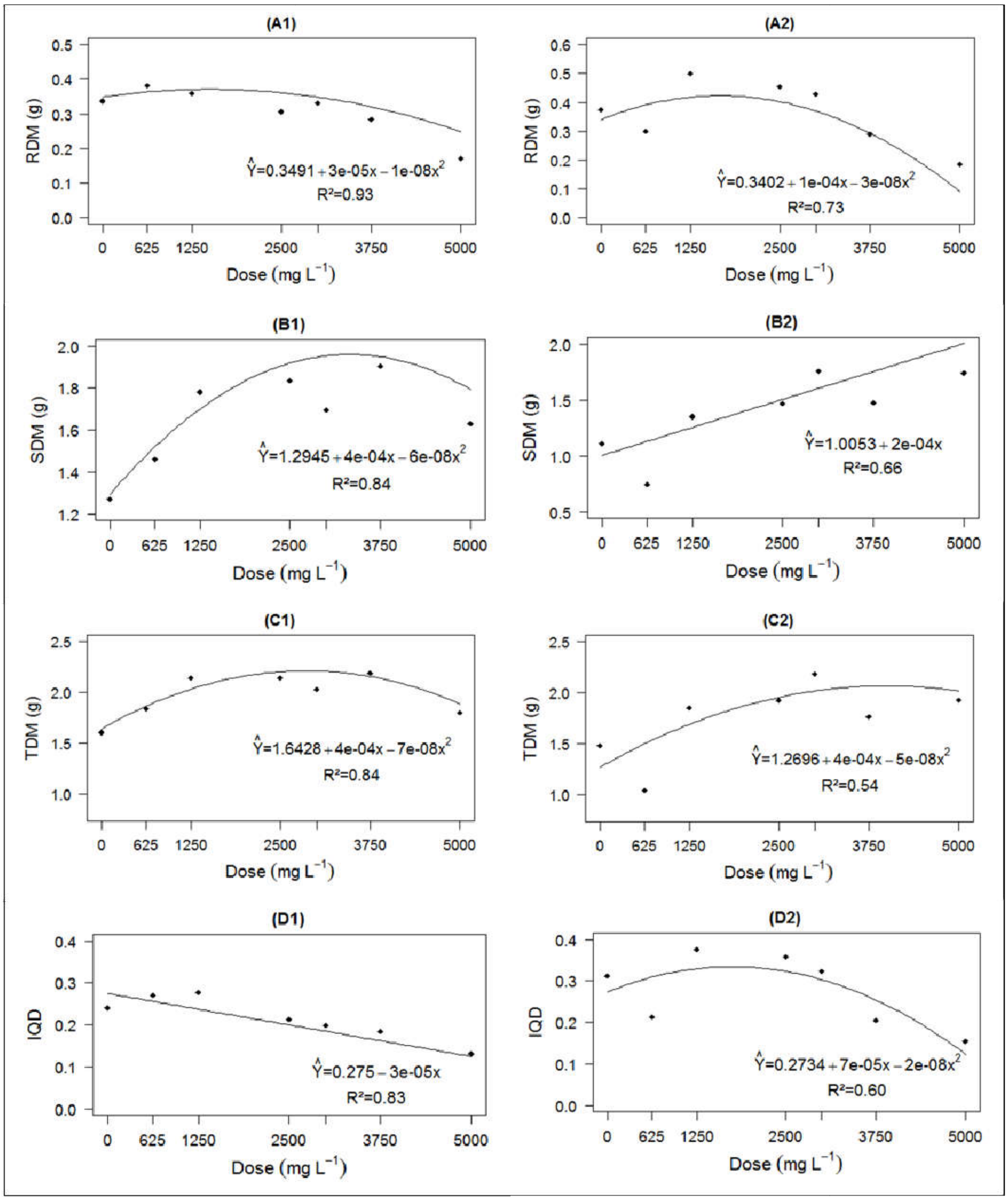

Fig. 2. Root dry matter mass (RDM) (A), shoot dry matter mass (SDM) (B), total dry matter mass (TDM) (C) and Dickson's quality index (IQD) (D) of plants produced with IBA diluted in water (1) and acetone (2) from cuttings at 150 days of age depending on the hormone doses applied

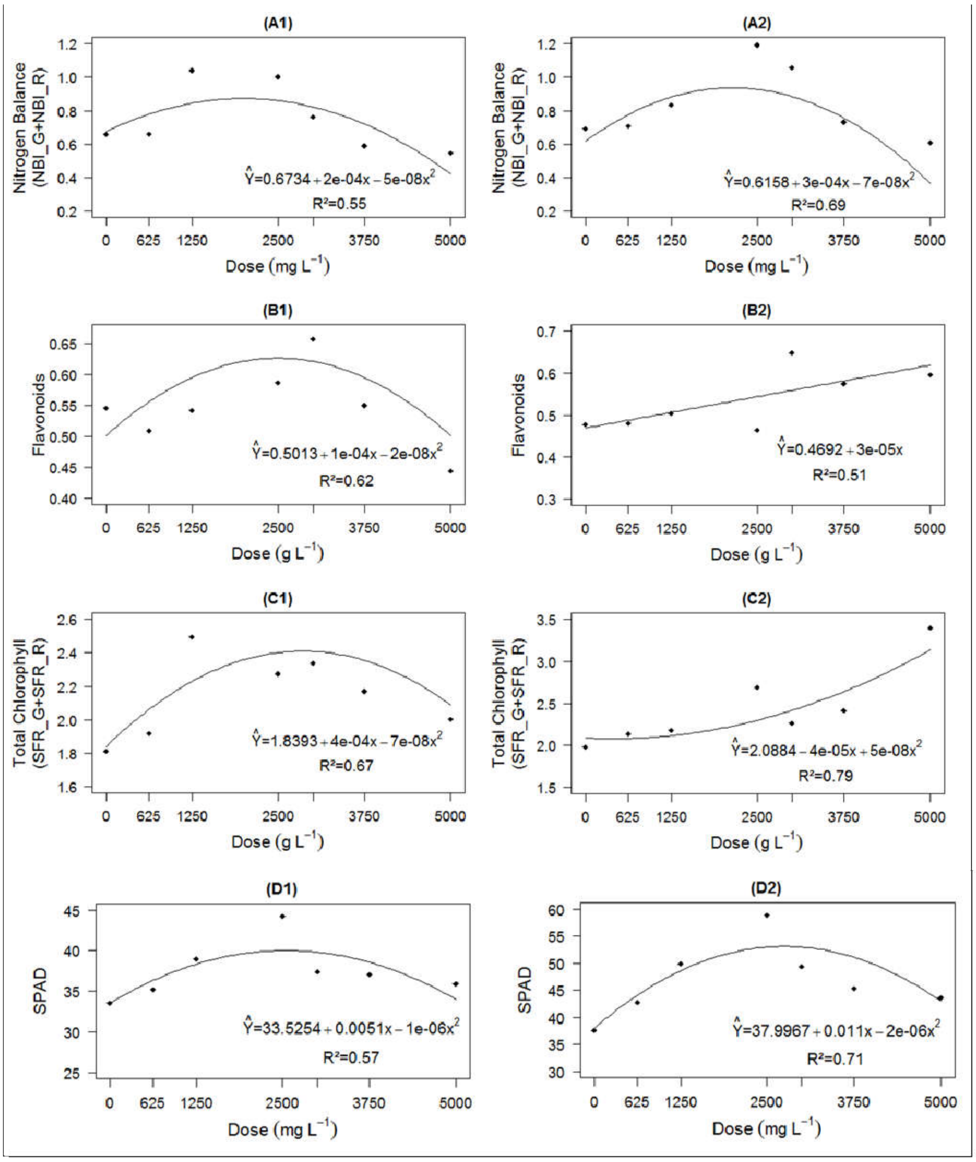

Fig. 3. Nitrogen balance (NBI_G + NBI_R) (A), flavonoids (B), total chlorophyll (SFR_G + SFR_R) (C) and SPAD index of plants produced with IBA diluted in water (1) and acetone (2) from cutting at 150 days of age depending on the hormone doses applied

The highest estimated IQD value was 0.33 obtained at a dose of 1750 mg/L for plant cuttings in which the IBA was diluted in acetone. This represents a 20.7% gain in plant quality in comparison to the treatment without using IBA. From this value, the IQD decreased with the increase in IBA doses (Fig. 2-D2). Hunt [24] established the minimum value for the IQD was 0.20 which is a good indicator of cutting quality in general, therefore, in this study, results were found to be higher than the minimum index established by Hunt. Sales *et al*. [25] studied the effect of different sources of organic matter in the propagation of *Schinus terebentifolia* Raddi, where they found IQD values that varied from 0.01 to 0.32 in plants after 121 days.

According to Bonamigo et al. [26], the IQD is essential to assess the quality of plant. It is an indicator of the performance of the cuttings, after are planted at their final location.

In the physiological variables of nitrogen balance (NBI_G + NBI_R) (Fig. 3-A1 and 3-A2) and

SPAD index (Fig. 3-D1 and 3-D2), in both forms of dilution of IBA (water or acetone), the inhibitory effect caused by IBA at high concentrations can also be observed. Regarding the nitrogen balance (NBI_G + NBI_R) the highest values were 0.87 and 0.94 with 2000 mg/L and 2142.86 mg/L of IBA in plants produced with IBA diluted in water and acetone, respectively. The SPAD index, which estimates the intensity of the green color of the leaves, increased to 40.03 and 53.12 with the use of 2550 mg/L and 2750 mg/L of IBA in cuttings submitted to treatment with IBA diluted in water and acetone, respectively. From these values, the increase in IBA concentrations caused damage to plants and caused a reduction in the nitrogen balance and the SPAD index.

The application of IBA increased the levels of auxin in plants and this can delay the onset of leaf abscission and increase the quantity of chlorophyll in the leaves, which contributes to the survival of cuttings, however, in high concentrations the IBA can be toxic and cause damage such as yellowing and leaf shedding, affecting the chlorophyll content and, consequently, the intensity of the green color of the leaves [27,28], thus, doses above the maximum point estimated have affected the SPAD index in aroeira cuttings.

4. CONCLUSION

In general, the preparation of IBA with acetone proved to be more efficient in the production of *Schinus terebentifolia* Raddi plants from cuttings when compared to IBA prepared with water.
The physiological characteristics were less favored in the aroeira plants where the IBA was applied with dilution in water. In this case, the results showed a growth reaching the maximum point, depending on the analyzed parameter.
The estimated dose of the 1750 mg/L of IBA prepared with acetone, made it possible to produce aroeira plants from cuttings better with quality index (IQD), whereas in the use of IBA prepared with water, there was a continuous tendency to reduction in quality index with the increase in the concentration of hormone.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors would like to thank the Federal Institute of Espírito Santo (IFES), the Espírito Santo Research and Innovation Foundation (FAPES) and the National Council for Scientific and Technological Development (CNPq) for the financial support and the nursery Florestar LTDA for making available the place to carry out the experiment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/69727